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16. ABSTRACT

Implimentation of the goals of each EPA regulatory program requires the use of laboratory test data to determine the toxicological properties of the chemical substances being regulated. This information is then used to predict the potential impact of a material on the environment. Since the implimentation of the "Acts", considerable research effort has been devoted to developing a wide variety of biological toxicity tests to define the effects of chemicals on organisms and communities. The purpose of this paper is to examine how various types of toxicological data are organized, interpreted and utilized in making regulatory decisions.

Two Approaches are examined in detail. First, the strategy used for developing water quality criteria for the protection of aquatic life. Second, the concept of hazard assessment is examined emphasizing both the similarities and differences with the criteria strategy. Finally, a retrospective case study is presented for each strategy to illustrate the relationship between the predictions derived from laboratory toxicity tests and field observations.

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INTRODUCTION

There are five major pieces of federal legislation that define the role of the Environmental Protection Agency with regard to marine and estuarine pollution: the Federal Water Pollution Control Act (1965); the Marine Protection, Research and Sanctuaries Act (1972); the Resource Conservation and Recovery Act (1976); the Toxic Substances Control Act (1976) and the Federal Insecticide, Fungicide and Rodenticide Act (1975). Each of these Acts charges the administrator of EPA with a variety of responsibilities that are generally summarized as follows: develop comprehensive programs to conserve waters for the propagation and protection of fish, aquatic life and wildlife; regulate the dumping of all types of materials into ocean waters and to prevent or strictly limit the dumping into ocean waters of any material which would adversely affect human health, the marine environment, ecological systems or economic potentialities; and to develop adequate data with respect to the effect of chemical substances and mixtures on health and the environment.

Implicit in the implimentation of the goals of each of these regulatory programs is the use of laboratory test data to determine the toxicological properties of the chemical substances being regulated. This information is then used to predict the potential impact of a material on the environment. Since the implimentation of the "Acts", considerable research effort has been devoted to developing a wide variety of laboratory toxicity tests designed to define the effects of chemicals on a variety of biological processes. A detailed analyses of these tests, their expected variances, and the degree of confidence in the types of data generated from these tests, has been reviewed in this volume by Hansen (1983).

The purpose of this paper is to examine how various types of toxicological

data are organized, interpreted and utilized in making regulatory decisions. To illustrate this, two approaches will be examined in detail. First, the strategy used for developing water quality criteria for the protection of aquatic life. Second, the concept of hazard assessment is examined emphasizing both the similarities and differences with the criteria strategy. Finally, a retrospective case study is presented for each strategy to illustrate the relationship between the predictions derived from laboratory toxicity tests and field observations.

Water Quality Criteria

A water quality criterion is a numerical value(s) that identifies the highest water concentration of a compound that will produce a water quality generally suited to the maintenance of aquatic life and its uses. Publication of water quality criteria has been an evolutionary and ongoing process involving EPA and its predecessor Agency, the Federal Water Pollution Control Administration since the publication of the first compilation of water quality criteria, the "Green Book" in 1968 (U.S. Dept. Interior, 1968). Since then, water quality criteria have been revised and expanded with the publication of the "Blue Book" (U.S. EPA, 1972) and the "Red Book" (U.S. EPA, 1976). Criteria published within this time period were characteristically derived by a panel of experts from very limited and non-quality assured data bases. In addition to the single, combined criteria for fresh- and salt-waters, the derivation of criteria often reflected an inconsistent use of data and a large measure of subjectivity. Furthermore, there was no consistent rationale for defining appropriate data and their use in deriving a criterion.

The general approach that characterizes this period (prior to 1976) is summarized in Figure 1. For pollutants whose bioavailability was

known to be influenced by water quality (e.g. hardness), the aquatic life criterion was derived by multiplying the LC50 (concentration lethal to 50% of the exposed population) of the sensitive important species tested in site water by a compound-specific, lab derived application factor. The term application factor is defined as the Maximum Acceptable Toxicant Concentration from a chronic test divided by the 96-hr LC50. In the case of non-water quality related chemicals (e.g. most pesticides) two procedures were available. First the lowest LC50 from the most sensitive species, was multiplied by a general application factor of 0.01 or alternatively, the lowest concentration not effecting survival, growth, or reproduction in a chronic test was utilized. The second procedure for developing criteria was applied to those chemicals that were known to bioaccumulate extensively and for which either FDA action levels for humans or wildlife effects studies were available. In this approach, the maximum permissible tissue concentration (FDA-action level), is divided by the highest bioconcentration factor [BCF = edible tissue concentration (mg/kg)/water concentration (mg/l)] to give an ambient water concentration that cannot be exceeded. The above procedures were used to calculate the maximum pollutant concentration that was deemed protective of aquatic life, and in the case of tissue residues, assured the marketability of fish and shellfish.

As mentioned previously these procedures suffered from the lack of an internally consistent rationale for selecting and interpreting data to eliminate subjectivity. Recognition of the need for scientific guidelines was reinforced when EPA was directed in 1977, via court order (NRDC, et al., V. Train, 1976) to publish criterion documents for 65 chemicals or groups of chemicals which Congress designated as toxic. To satisfy this

legal mandate a systematic toxicological testing program was instituted to develop comprehensive data bases on freshwater and saltwater species and a task force of EPA scientists was constituted to develop the scientific rationale that forms the basis for developing water quality criteria. The Guidelines for the Development of Water Quality Criteria (U.S. EPA, 1980); represents a rationale that describes the collection, review, selection and categorizing of toxicological information. The categories include information on acute and chronic toxicity to animals and plants, bioaccumulation data, and other data such as that derived from studies on physiology, biochemistry, community structure, function and bioaccumulation. The Guidelines also define minimum data requirements in the various categories that are necessary to formulate criteria and prescribe methods for using these data in the formulation of criteria. Consequently, the Guidelines provide an internally consistent rationale and well defined procedures for deriving criteria that eliminate the problems of subjectivity inherent in the earlier process. This, coupled with a systematic and quality assured data base, represents a major advancement in the application of toxicological information to the regulatory process. The intent of this rationale and the resulting criteria is the protection of most of the species most of the time but not all the species all of the time. Further, it assumes that if most species are protection, maintenance of community function will be assured.

Numerical criteria derived using the current Guidelines are expressed as two numbers: a maximum concentration, and a 30-day average concentration (Figure 2). The two-number criterion is intended to identify the highest average concentration consistent with the maintenance of aquatic life while restricting the extent and duration of excursions over the average

so that the total exposure will not cause unacceptable adverse effects.

The maximum component of the criterion is designed to limit acute lethality and is based upon the compilation and analysis of acute toxicity data from a minimum of eight taxonomic saltwater families. Specifically, for a saltwater criterion, acute toxicity data from the following taxa are required: two different families in the phylum Chordata, a family in a phylum other than Chordata and Arthropoda, either the Mysidae or Penaeidae family, three other families not in the phylum Chordata, and any other family. The species acute toxicity data are arranged in families which are then ranked from most to least sensitive and regression analysis employed to determine the Final Acute Value (Erickson and Stephan, 1982).

The Final Acute Value, which is an estimated LC50, is multiplied by 0.5 to give the Maximum Concentration which is an estimated LC0 or nonlethal concentration. This value is intended to protect 95% of a group of families with similar sensitivity as those tested when the cumulative deviation of excursions above the specified 30-day average concentration is limited to 96 hours in any 30 consecutive days. Inherent in the rationale described above for determining the Maximum Concentration are the following assumptions: a) the range of species sensitivity from laboratory tests is an unbiased estimator of the range of sensitivity of species in natural communities; b) the variability of species within families is less than between species across higher taxonomic groups; c) that the ranking of family sensitivities of laboratory populations is not different from that of field populations; d) laboratory test data is not intrinsically biased, though never originally chosen to provide a random reflection of the species in the environment; e) the maximum concentration is designed to protect most of the species most of the

time; f) by protecting species composition, community function is protected.

Derivation of the 30-day average component of a criterion involves the interpretation of data bases on chronic toxicity to animals, toxicity to plants, bioaccumulation, and other biologically significant effects on ecologically or economically important resource populations (Figure 2).

There are two methods for determining chronic toxicity to animals. The first option is to perform a regression analysis on eight families of chronic tests. Usually, this option is not practical because of the limited number of chronically testable saltwater species and the prohibitive cost of such testing. The second option utilizes the acute-chronic ratio concept. To determine an acute-chronic ratio for a given species a measure of acute toxicity, defined as a ninety-six hour LC50, is divided by a measure of chronic toxicity, defined as the geometric mean of the highest no effect and lowest effect concentration. Effect and no effect are defined as being statisticallly different or indistinguishable from appropriate controls. The guidelines recommend that acute-chronic ratios from species representing a minimum of three families one must be saltwater and must have a fish and an invertebrate as being a satisfactory estimator of the relationship between acute and chronic effects. The geometric mean of these acute-chronic ratios is divided into the Final Acute Value in order to provide a chronic exposure concentration protective of untested species. The assumptions inherent in this approach are that acute-chronic ratios from laboratory tests are an unbiased estimator of those expected from natural populations. Further, for certain compounds the acute-chronic ratio is similar across species and consequently can be used to extrapolate to untested species. In contrast, the acute-chronic ratio for some compounds decreases with increasing species sensitivity and this

relationship has been used to predict chronic sensitivities for untested species. Since there is often two or more orders of magnitude difference in the range of acute toxicity it is more important to have a good estimate of the range of species acute sensitivities, to which the acute-chronic ratio can be applied (Hansen, 1983), than to have a large number of acute-chronic ratios.

The Final Residue Value (Figure 2) is utilized in developing criteria to protect marketability by preventing commercially or recreationally important aquatic organisms from containing amounts of chemicals exceeding relevant FDA action levels (maximum permissible tissue concentrations) and protect wildlife, including fishes and birds that eat aquatic organisms. This concept is not unique to the current Guidelines and, in fact, has been utilized in limited cases in previous criterion documents. What is different is the quality assurance and minimum data requirements. In addition, for lipophilic material the bioconcentration factor (BCF); the quotient of the concentration of a substance in all or part of an aquatic organism divided by the concentration in water to which the organism has been exposed, is normalized to 1% lipid. This adjustment seeks to make all the measured BCF's comparable regardless of the species or tissue for which the BCF was measured. The final residue value is derived by dividing the maximum permissible tissue concentration by an appropriate BCF and multiplying this by the greatest percent lipid in a marketable species. The final residue, adjusted for highest lipid, defines the pollutant concentration in the water that cannot be exceeded to protect marketability or wildlife consumers of aquatic life.

The remaining categories of data that can contribute to estimating the chronic 30-day average component of the criteria are plant data and

other data, including physiological, biochemical and behavioral data on ecologically or commercially important species and data on impacts on community structure and function. Information in these two categories are generally supportive of criteria derived from chronic animal tests and residue values but have not been the basis for criteria derivation in of themselves.

The derivation of the 30-day average criterion is based upon the systematic comparison of data from all four categories of chronic tests and the selection of the lowest value. This value is deemed protective of aquatic life from chronic effects, and when coupled with the Maximum Value to protect for acute effects, provides the scientific information necessary for this specific management decision. Thus recommendations of chemically specific water quality criteria derived by using the current Guidelines are based upon: a) a defined and consistent rationale for utilizing all available pertinent data; b) quality assured data bases; c) minimum data requirements for all information categories d) analysis of data using statistical procedures.

The Guidelines procedure we've been describing is utilized to predict the water concentration of a chemical that is suited to the maintenance of aquatic life. Another way of viewing the Guidelines then is as a predictive method that is based on the synthesis and interpretation of toxicological data. Prediction is the key to the regulatory process since EPA is mandated to make decisions on the registration and disposal of chemicals prior to their use (TSCA-Section 4, Premanufacture Notice/ Pesticide Registration). Having developed a predictive rationale such as the Guidelines, the next step is to evaluate the accuracy of its predictions. This involves a field applicability or verification study.

We do not have, unfortunately, a direct field verification study in marine waters for the current guidelines. We do have however a freshwater field case study, Shayler Run, that tested the principals of the current guidelines and provides insights into the limits of applicability of laboratory studies for predicting responses in the field, that is, a measure of field verification.

Shayler Run

The Shayler Run study 1967-1972 (Geckler et al., 1976) was designed to evaluate the usefulness of laboratory toxicity tests in predicting water quality criteria for protection of freshwater aquatic life. The study was designed so that the results from standard laboratory and streamside acute and chronic toxicity tests could be compared with the results of long-term stream exposure. The field verification portion of this study lasted four years and nine months. The exposure concentration in the stream was selected using acute toxicity data for ten fish species and chronic toxicity data for fathead minnows so that the most resistant fish species would be protected from chronic effects and the most sensitive would not be protected. Since this study predates the current guidelines it is not intended as a direct verification of the latter. It does however provide insight into the appropriateness of many of the concepts and assumptions in the current guidelines and is useful therefore in assessing their validity.

The regulatory climate that prevailed at the time of the study reflected the 1965 Federal Water Pollution Control Act which required water quality standards to protect designated uses. Uses could be defined, for example, as the maintenance of a salmonid, warmwater sport or rough-fish fishery, maintenance for recreational uses or merely for navigation.

Within this context, broader ecological concepts such as community structure and function and species diversity were not specifically studied. The objectives and hypotheses implicit within the Shayler Run study are: a) the conditions acceptable for the designated use also provide satisfactory conditions for the supporting ecosystem components (e.g. food species), b) the exposure-response relationships determined for individual species using laboratory effect measurements are not different from responses determined in the field for the same species; c) the relative sensitivity of the same species is not different in lab tests and in the field; d) indirect effects due to the toxicant on other components of the field system are not significant in altering the responses of the test species; e) the laboratory derived acute-chronic ratio can be used to protect fishes in the field.

The experimental design for the Shayler Run studies consisted of three components: (1) acute and chronic toxicity test in standard laboratory water; (2) streamside acute and chronic tests with stream species, and (3) field studies. Acute toxicity tests were conducted to determine the effect of changes in the water quality of Shayler Run on toxicity and the relative sensitivity of ten fish species common in the stream. Chronic toxicity studies were used to define the copper concentrations affecting survival, growth and reproduction in fathead minnows and to measure, the ratio of acute to chronic toxicity. This ratio, was then applied to the acute tests for the other 10 fish species in Shayler Run water, to predict safe concentrations for untested species. These values were used to select the copper field exposure concentration of 120 $\mu g\ l^{-1}$. Field studies included eight biannual (spring and fall) samplings of fish populations using electric seining techniques, daily collections of

vertebrates and invertebrates from weir screens, and fish feeding patterns from analysis of stomach contents. Reproduction and young of the year growth rates were estimated from fry collection traps and weirs and from direct spawning observations. Nonfish studies included the examination of benthic macroinvertebrates and periphyton communities.

The results of the field study indicated that, as predicted, the most sensitive fish species in Shayler Run were adversely affected by copper. However, with one exception, copper adversely affected almost every common species of fish that spawned in the exposure and recovery areas by restricting spawning locations. Generally, spawning occured within reaches of the exposure portion of the stream where copper concentrations were 60-90 $\mu g\ l^{-1}$ (a gradient of copper concentrations commonly existed in the 900 meter exposure area). Four of the five most abundant macroinvertebrate populations in the exposure area were eliminated by copper. Although dietary shifts were obseved in two of the fish species, indirect effects on growth of the fish populations through effects on the aquatic food chain could not be demonstrated.

We have discussed this study because it is an indirect field verification of several of the concepts and hypotheses implicit in the current Guidelines for developing water quality criteria. The conclusions that can be drawn from this study are totally consistent with our original hypothesis. The dose-response relationships and sensitivity ranking of resident fish species in laboratory tests were within a factor of two of those determined in the field for the same species. The greater sensitivity in the field was due to field-observed avoidance to concentrations of copper between 90 and 120 $\mu g/l$, a factor not accounted for during standard toxicity tests. Secondly, indirect ecological effects (e.g.

dietary shifts) did not alter the field responses. The fact that results of laboratory toxicity tests, within the context of the experimental design, were within a factor of two of the field observations is both remarkable and yet it totally consistent with the expected variability in toxicological tests conducted under the most rigid laboratory controlled conditions (Hansen, 1983). Regarding the second hypothesis, concentrations of copper determined to be protective of the organisms of interest (e.g. blugill and other sunfish) were not protective of many species of macro-invertebrate or other ecosystem characteristics. Because the experimental design did not include toxicity information on other ecosystem components (e.g. invertebrates, plants, etc) their sensitivities were not accounted for and thus the degree of protection afforded to these groups was not determined. This study also provides a first order estimate of the confidence of our laboratory predictions, and identifies a series of issues that would further enhance the credibility of laboratory data. Recently, Hansen and Garton (1982) assessed the ability of a standard set of freshwater single-species toxicity tests to predict accurately the effects of an insecticide on complex laboratory stream communities. In this study, the single-species tests adequately predicted the concentrations of the insecticide, diflubenzuron, that affected the stream communities but were less successful in predicting the exact nature of the community level effects. Those effects resulting from direct lethality to component species were clearly predicted; indirect effects due to altered interspecies interactions could only be predicted with an a priori knowledge of the system's trophic dynamics. What is important is that the strategy of defining a wide range of species sensitivities from acute tests and coupling them with acute-chronic ratios can be a highly accurate predictive

tool when properly applied. Within the context of the needs of the regulatory process, I would offer that the Guidelines constitutes a strategy that is in effect a very "Meaningful Measure of Marine Pollution". This is a particularly important point to understand. We are not talking of a single test method any longer but a rationale for organizing and interpreting a variety of test data from which we can derive predictions with a specified degree of confidence.

Hazard Assessment

The enactment of the Toxic Substances Control Act (1976) provides that no person may manufacture a new chemical substance or manufacture or process a chemical substance for a new use without obtaining clearance from the EPA. TSCA represents an attempt to establish a mechanism whereby the hazard to human health and the environment of a chemical substance is assessed before it is introduced into the environment (Cairns, et al., 1978). The enactment of TSCA provides the stimulus for both government and industry to develop testing and decision making procedures that represent a systematic and comprehensive approach to the problem of predicting chemical hazard to the environment. The initial premise of aquatic hazard evaluation procedures is that toxicological data are interpreted in relation to the expected or measured exposure concentration of the material in the aquatic environment (Kimerle et al., 1978). This leads to a description of the relationship between the generic types of information that form the basis for a hazard assessment. It is important to point out at this time that the Water Quality Criteria and the Hazard Assessment Strategies both utilize laboratory data on aquatic toxicity. Hazard Assessment, however, requires additional laboratory information necessary to predict chemical concentrations in the environment. This

latter requirement is one of the principal differences between these two approaches and determines how they are applied in the regulatory process. The other difference relates to the amount of information required of each strategy to make a decision. The criteria rationale requires very comprehensive minimum data requirements to derive a prediction because these chemicals have been identified as hazardous. Hazard assessment does not require as structured a data set, instead the testing is designed to be sequential. Moving between each level or hierarchy of testing is controlled by specific criteria. Decisions can be made at each level based on varying amounts and complexities of information. The details of hazard assessment are available in an excellent review by Cairns et al., 1978. For the purposes of this paper we will focus on the types of biological test data used in hazard assessment and illustrate how this is coupled with predicted or measured exposure concentrations to determine potential hazard. Two case studies representing major environmental problems, will be retrospectively analyzed to illustrate the applicability of this approach as a meaningful measure and predictor of marine pollution.

Case Histories

Few case histories exist that describe: (1) the release of a chemical contaminant from a point source into an estuarine environment; (2) the subsequent monitoring of the pollutant; and (3) the correlation of the field data with a substantial toxicity data base. Two such case histories are the presence of polychlorinated biphenyl (PCB) Aroclor® 1254 in Pensacola Bay, Florida, and of the insecticide Kepone® in the James River, Virginia estuary.

Aroclor® 1254

In the case of Aroclor® 1254, an industrial plant released a quantity of the chemical into the Escambia River (Duke et al., 1970). Residues of Aroclor® 1254 contaminated the biota and sediment of the bay system. Due to the toxic, persistent and bioaccumulative nature of PCBs, and their presence in most aquatic habitats worldwide, these chemicals were added to the U.S. Environmental Protection Agency's (EPA) Consent Decree Chemical list (U.S. EPA, 1980a) and as such provided the data for generating a water quality criterion for PCBs, (U.S. EPA, 1980b).

Figure 3 illustrates much of the available marine toxicity data base on PCBs. Data for the acute toxicity screening tests encompass the Aroclors® 1016, 1248, 1254 and 1260. The acute toxicity data base should be interpreted with caution, however, because the solubility of PCBs is frequently exceeded in these tests. Of the six data points, only one (96hr EC50 as shell deposition in the eastern oyster, Crassostrea virginica, 14 µg/l) was for Aroclor® 1254 (Personal communication, J.I. Lowe, U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida). Oyster shell deposition studies with other PCBs provided EC50 values that were within a factor of 6 of the Aroclor® 1254 value (10.2 µg/l, Hansen et al., 1974; to 60 µg/l, personal communication, J.I. Lowe). The 96-hr LC50 values for brown shrimp, Penaeus aztecus, and grass shrimp, Palaemonetes pugio, exposed to Aroclor® 1016 were also within the range observed with the oysters (10.5 and 12.5 µg/l, respectively; Hansen et al., 1974).

Several long-term exposures of juvenile or adult marine animals to PCBs were conducted. Hansen et al. (1971) exposed juvenile spot, Leiostomus xanthurus, and pinfish, Lagodon rhomboides, to 5 µg/l of the PCB for 14 to 45 days. They reported significant incidences of hemorrhagic

lesions and death in both species. Nimmo et al., (1971) exposed pink shrimp, Penaeus duorarum, to Aroclor® 1254 for 15 days; they reported 51% mortality in animals exposed to 0.94 µg/l of the chemical. A second study by Nimmo and Bahner (1976) reported a similar value (1.0 µg/l) as the 15-day LC50 of pink shrimp.

Only two early life stage toxicity tests were conducted on marine animals with Aroclor® 1254; both involved the sheepshead minnow, Cyprindon variegatus. Schimmel et al. (1974) reported a 21-day LC50 of 0.93 µg/l for embryos and larvae of the species; the maximum acceptable toxicant concentration (MATC) was 0.098 µg/l. Hansen et al. (1973) exposed adult sheepshead minnows to Aroclor® 1254 for four weeks, injected the females for egg production, and held the subsequent embryos and larvae in PCB-free water for an additional four weeks. They reported that survival of the early life stage was significantly reduced when adult fish were exposed in a measured PCB concentration of 0.14 µ g/l; no significant effects were observed in progeny from adults exposed to 0.09 µg/l. In this instance mortalities were caused by PCB within the eggs greater than 7 mg/kg. This body-burden related effect is seldom observed and would have been overlooked in this test had not the animals been placed in clean sea water.

The last data point on Figure 3 refers to a settling community study by Hansen (1974). In that study, unfiltered sea water received three concentrations of Aroclor® 1254 and flowed over clean sand in several aquaria. Planktonic larval stages of estuarine animals in the sea water settled and grew in the aquaria over a four-month period. Significant differences in community composition were observed in aquaria receiving PCB concentrations $\geq$ 0.6 µg/l.

As illustrated in Figure 3, the increasing complexity of testing, the longer periods of exposure, and the testing of the more sensitive portions of the life cycle provide lower effect concentrations. It is evident that the acute toxicity tests would not predict the adverse chronic effects such as observed in fish early life-stage toxicity tests. Indeed, the acute toxicity of Aroclor® 1254 is more than two orders of magnitude greater than its chronic toxicity (Figure 3). The similarity of the concentrations of PCBs measured in Escambia Bay, Florida (0.07 to < 0.05 µg/l; Schimmel et al., 1974 and Nimmo et al., 1971) to those producing adverse chronic effects in the laboratory (0.098 µg/l; Schimmel et al., 1974), was close enough to cause concern.

For those chemicals that have been assigned an FDA action limit, one can also establish an isopleth based on the maximum permissable tissue concentration (MPTC; U.S. EPA, 1980a). An MPTC isopleth for Aroclor® 1254 (5.0 mg/kg) is illustrated in Figure 3. The amount of any chemical taken up from water into tissues of aquatic animals is dependent upon factors such as the chemicals concentration in water, its octanol/water partition coefficient, the duration of exposure, gill ventilation rates of the exposed animal, etc. Bioconcentration studies with Aroclor® 1254 using various estuarine species, exposure concentrations, and durations of exposure indicate that the FDA action level can be exceeded in spot exposed to 1.0 µg/l of the PCB in seven days (Hansen et al., 1971). The longer the exposure, the more PCBs are accumulated until steady state is reached. Female sheepshead minnows exposed to 0.09 µg/l for 28 days accumulated PCBs to one half the action level (Hansen et al., 1973) while oysters, C. virginica, exposed for 56 days to 0.005 µg/l (measured concentratons) accumulated to 1.65 mg/kg or 33% of the action

level (Parrish et al., 1974). In another study (Lowe et al., 1972), oysters exposed to 10 µg/l Aroclor® 1254 for 24 weeks concentrated the chemical in their tissues 101,000 X the exposure concentration (20 X the action level). It is obvious from the MPTC isopleth, that an exposure concentrations measured in Escambia Bay, Florida could result in residues approaching or exceeding the action level and in fact they did (Nimmo et al., 1971).

Kepone®

Release of the chlorinated insecticide Kepone® (chlordecone) into the James River, Virginia in the 1960's and 70's, and the subsequent contamination of estuarine water, sediment and biota is well documented (Onishi and Ecker, 1978; Bender et al., 1977; Hugggett et al., 1980). Kepone® concentrations in James River oysters were varied by season averaging 0.16 mg/kg at eight locations; amounts in finfish averaged from 2.7 mg/kg for white perch (a long-term resident) to 0.81 mg/kg for spot, a short-term resident (Bender, et al., 1977). Kepone® concentrations in sediment varied considerably but were related to the proximity from the source of the release (Hopewell, Virginia), and correlated with sediment organic content (Nichols and Trotman, 1977). Dissolved Kepone® in water samples collected from the estuarine portion of the James River averaged 0.01 µg/l (Onishi and Ecker, 1978).

A substantial laboratory data base exists on the acute toxicity of Kepone® to estuarine animals (Figure 4). The 96-hr LC50 for Mysidopsis bahia was 10.1 µg/l (Nimmo et al., 1977), and that for the blue crab Callinectes sapidus was > 210 µg/l (Schimmel and Wilson, 1977). Estuarine finfish 96-hr LC50 values for six species ranged from 6.6 µg/l for the spot to 69.5 µg/l for sheepshead minnows (Butler, 1963; Schimmel and

Wilson, 1977; Roberts and Bendl, 1982).

Three studies were conducted involving the sensitive life stages of estuarine animals (Nimmo et al., 1977; Hansen et al., 1977 and Goodman et al, 1982). Nimmo et al. (1977) conducted a full life cycle toxicity test with M. bahia and reported that the MATC (based on reproductive impairment) was less than 0.39 µg/l, the lowest concentration tested. In a separate test, juvenile M. bahia were exposed to five concentrations of Kepone® for 14 days. They reported that growth was affected at 0.075 µg/l but not at 0.026 µg/l, giving an MATC of 0.043 (Figure 4). Hansen et al., (1977) conducted toxicity tests using various life stages of the sheepshead minnow. Results of their studies indicated that the 28-day LC50 for adult fish was 1.3 µg/l. However, growth of juvenile progeny in that study was significantly reduced by exposure to 0.08 µg/l Kepone®, the lowest concentration tested. Therefore, the calculated MATC would be less than 0.08 µg/l (Figure 4). Goodman et al., (1982) conducted an entire life-cycle toxicity test with sheepshead minnow. the most sensitive endpoint in the study was growth of the progeny at 0.12 µg/l Kepone®. The calculated MATC was 0.094 (geometric mean of 0.074 and 0.12 µg/l).

As with the data shown with PCBs, it is evident that by increasing the duration of exposure to Kepone®, by using more sensitive life stages and more discriminatory endpoints, the chronic toxicity is revealed. In doing so, we can better define the ultimate risks to estuarine species. In the case of Kepone®, we see that growth for both mysid shrimp and sheepshead minnows was affected at Kepone® concentrations between 0.04 and 0.08 µg/l; the best estimate of Kepone® concentrations in the James River is 0.01 µg/l. The small difference between the two values suggests

a relatively high probability of adverse biological effects associated with Kepone® in the James River.

As with PCBs, the U.S. Food and Drug Administration (FDA) has assigned an action level (MPTC) for Kepone® in fish and shellfish (0.3 mg/kg). Laboratory studies and chemical analysis of aquatic life in the James River prove the significant bioaccumulative properties of Kepone®. In laboratory studies, sheepshead minnows surviving 96-h exposures bioconcentrated Kepone® as high as 1,548 times the concentration measured in the exposure water (Schimmel et al., 1977). Given an MPTC of 0.3 mg/kg, the maximum acute exposure of Kepone® allowed to this fish would be 0.000194 mg/l or 0.194 µg/l (0.3 ÷ 1,548). Results of longer term bioconcentration studies at or near steady-state indicate higher bioconcentration factors (BCF). Bahner et al. (1977) exposed C. virginica to Kepone® and reported BCF values of approximately 9,300x in 19 days, while the crustaceans, M. bahia and P. pugio, bioconcentrated 13,473 and 11,425 in 21 and 28 days, respectively. The BCF of Kepone® in fish at steady-state was lower than that shown by invertebrate species (7,100x for C. variegatus after a 30-day exposure; Bahner et al. (1977). Using the highest BCF generated, (13,473x for mysids) and the 0.3 mg/kg action level, we calculate that a maximum, steady-state exposure of 0.02 µg/l could produce tissue concentrations exceeding the MPTC. This water concentration is only a factor of two greater than that measured in the James River (Onishi and Ecker, 1978) and certain species contained Kepone® concentrations in excess of the MPTC.

Discussion

Implicit in hazard assessment are several concepts: (1) there is a coupling of the biological effects observed and the environmental exposure

expected; (2) the procedure is predictive in its intent, not post facto monitoring, and (3) the monitoring aspect is a feedback procedure to verify or compare the results with the prediction.

The two estuarine case histories, are obviously post facto observations of environmental contamination. The purpose of their presentations here is to illustrate that within the context of a thorough hazard assessment, and strict adherence to the recommendations derived from that assessment, neither episode would likely have occurred. In the case of PCBs, several properties of the chemical should have provided clues to it's ultimate hazard. First, the higher chlorinated PCBs, including Aroclor® 1254, have very high octanol/water partition coefficients and as such would be expected concentrate in biological tissues (e.g. lipids). This single test (octanol/water partition determination) is a valuable first level test in identifying chemical hazard though it's bioaccumulation potential. Secondly, simple hydrolysis and photolysis studies would indicate negligible degradation through those pathways. Results of Aroclor® 1254 laboratory bioconcentration studies indicated a steady-state BCF of over 100,000 times (Lowe et al., 1972). When combined with the FDA action limit of 5.0 mg/kg, the maximum allowable PCBs in seawater should not exceed 0.05 µg/l. Results of field-derived BCFs indicate that the field BCF is greater than 670,000 times for spotted seatrout, Cynoscion nebulosus (Nimmo et al., 1971). This difference between the laboratory and field-derived values may be due to inaccurate estimates of PCBs in Escambia Bay waters or possibly an underestimation of the importance of aquatic food chains in the accumulation of PCBs. The inability to differentiate those chemicals that bioconcentrate from those that bioconcentrate and accumulate substantially within food chains, (or the lack

of appreciation of some chemical to do so) may be the weakest aspect of hazard assessment, and deserve more thorough evaluations.

On the effects side of hazard assessment, PCBs have moderately high acute toxicity ranging between 10 and 100 µg/l. More important, are the effects on biota in tests of longer duration, specifically, the effects on energetics, growth, and reproduction. We can see from Figure 3 that the lowest value producing adverse biological effects (0.098 µg/l in a C. variegatus early life stage test; Schimmel et al., 1977) was close to the range of environmental water concentrations in Escambia Bay, and that there probably existed no margin of safety. Indeed, based on their known insensitivity to other pollutants it would be naive to assume that the sheepshead minnow would be the most sensitive of all the species resident in the Bay. Had PCBs been subjected to the rigors of a hazard assessment process, responsible decisionmakers would have concluded that PCBs should not be allowed to approach environmental concentrations that would: be expected to produce tissue concentrations exceeding the MATC (0.05 µg/l); or adversely affect fishes (0.098 µg/l). The amount of safety margin (risk) they would apply to PCBs before manufacture would depend upon these data and the myriad of social and economic isues relating to the specific chemical under evluation, and the resources at risk.

The Kepone® case history contains many of the same clues that should allow the hazard assessment process to identify the potential threats of this insecticide to estuarine systems and infact this is why this chemical was not registered for use in this country. Careful exposure assessment through laboratory studies would identify several characteristics of Kepone® including: (1) Kepone®'s extreme refractory nature (Bourquin et al., 1978: Garnas et al., 1977); (2) its' strong affinity for sediments,

particularly the organic constituents (Nichols and Trotman, 1970; Huggett and Bender, 1980; Garnas et al., 1978); (3) low volatility (Dawson et al., 1978); and (4) high bioaccumulative capacity as determined by the log of the octanol/water partition coefficient (5.7, Personal communication, R.L. Garnas, U.S. EPA National Enforcement Investigations Center, Denver Colorado) and laboratory BCF studies (Bahner et al., 1977; Schimmel and Wilson, 1977, Hansen et al., 1977). Results of these data predict that its release into an estuarine system would contaminate water, sediment and biota of all trophic levels. Degradation of the material would be negligible as would loss due to volatilization. Therefore, loss of the chemical from the aquatic system could be extremely slow, possibly taking decades.

Similar to the exposure assessment, the effects assessment scheme should predict the hazard of Kepone® in estuarine environments. Kepone® was toxic to a wide variety of organisms including plants, crustaceans, molluscs, and finfish (Walsh, 1977; Schimmel and Wilson, 1977; Butler, 1963; Hansen et al., 1977; Nimmo et al., 1977; Roberts and Bendl, 1980). The ratio of the acute effect concentrations to the chronic effect concentrations ranged from 235X (Nimmo et al., 1977); to greater than 862X (Hansen et al., 1977). The necessity for conducting chronic tests in hazard assessment using environmental fate-triggers is obvious.

One unexpected result of the exposure assessment procedure was that of the accumulation of Kepone® in James River blue crabs. Schimmel and Wilson (1977) exposed blue crabs to Kepone® in acute tests and reported that the BCF in that period was approximately 8 compared to a high of 1,548 for sheepshead minnows. Male blue crabs collected from the James River, however contained an average of 0.81 mg/kg; females contained an average

of 0.19 mg/kg (Bender et al., 1977). One hypothesis for explaining the difference between low laboratory residues and significant Kepone® residues in field-collected blue crabs was that the crab's food may be a major source of Kepone®. Schimmel et al. (1979) tested this hypothesis and reported that indeed food (oysters) was the major contributing factor for Kepone® residues. In addition, crabs that accumulated approximately 0.07 mg/kg Kepone® suffered significant mortality and molted less frequently than those fed Kepone® free oysters. Studies by others were attempted to reproduce these effects, but were unsuccessful and were never published in the peer-reviewed literature. The adverse effects due to body burden and the contribution of food to that body burden were not expected and would most likely be overlooked in the hazard assessment protocol.

It is clear in the case histories illustrated here that the hazard assessment scheme identifies many of the properties that are potentially deleterious to the aquatic environment. One hazard that was not identified in both case histories is that related to probable importance of food chain transfer and the resultant deleterious effect of those body burdens. It is likely that only a few chemicals would respond like PCBs and Kepone® in this respect. However, we must learn from post facto exposures such as those illustrated here and should develop definitive predictive tests, or adapt existing tests to identify hazards that may not be predicted.

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References

Bahner, L.H., A.J. Wilson, Jr., J.M. Sheppard, L.R. Goodman, G.E. Walsh, and J.M. Patrick, Jr. 1977. Kepone® bioconcentration, accumulation loss, and transfer through estuarine food chains. Chesapeake Sci. 18:299-308.

Bender, M.E., R.J. Huggett and W.J. Hargis. 1977. Kepone® in Chesapeake Bay Biota. Proceeding of the Kepone® II Seminar. Easton, Maryland, Sept. 19-21, U.S. EPA Region III, Philadelphia, Pennsylvania 14-37.

Bourquin, A.W., P.H. Pritchard, and W.R. Mahaffey. 1977. Effects of Kepone® on estuarine microorganisms. Devel. Indus. Micro. 19:489-497.

Butler, P.A. 1963. Pesticide-Wildlife Studies - A Review of Fish and Wildlife Service Investigations during 1961 and 1962. U.S. Fish and Wildlife Circular. 167:11-25.

Cairns, J. Jr., K.L. Dickson, and A.W. Maki. 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. American Society for Testing Materials. STP 657.

Dawson, G.W., S.J. Shupe, and W.C. Weimer. 1978. Chapter IV Properties of Kepone. In The Feasibility of Mitigating Kepone Contamination in the James River Basen. U.S. Dept. Energy Contract EY-76-C-06-1830.

Duke, T.W., J.I. Lowe, and A.J. Wilson, Jr. 1970. A Polychlorinated Biphenyl (Aroclor® 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5:171-180.

Erickson, R.J. and C.E. Stephan. Manuscript, Calculation of the Final Acute Value for water quality criteria for aquatic life. U.S. EPA, Environmental Research Laboratory, Duluth, MN.

References (Cont'd)

Garnas, R.L., A.W. Bourquin, and P.H. Pritchard. 1978a. The Fate and degradation of 14C-Kepone® in estuarine microcosms. Contribution Number 351, EPA-Gulf Breeze Environmental Research Laboratory 22p.

Garnas, R.L., A.W. Bourquin, and P.H.. Pritchard. 1978. The Fate 14C-Kepone® in Estuarine Microcosms. Abstract for the American Chemical Society National Meeting, March 1978. Anaheim, California.

Goodman, L.R., D.J. Hansen, C.S. Manning, and L.F. Fass. 1982. Effects of Kepone® on the sheepshead minnow in an entire life-cycle toxicity test. Arch. Environm. Contam. Toxicol. 11,335-342.

Geckler, J.R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering, E.R. Robinson, C.E. Stephan. 1976. Ecological Researchh Series, EPA-600/3-76-116.

Hansen, D.J., P.R. Parrish, J.I. Lowe, A.J. Wilson Jr., and P.D. Wilson. 1971. Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes. Bull. Environ. Contam. Toxicol. 6:113-119.

Hansen, D.J., S.C. Schimmel, and J. Forester. 1973. Aroclor® 1254 in Eggs of Sheepshead Minnows: Effect on Fertilization Success and Survival of Embryos and Fry. Proc. 27th Annu. Conf. S.E. Assoc. Gam Fish Comm. 420-426.

Hansen, D.J. 1974. Aroclor® 1254: Effect on Composition of Developing Estuarine Animal Communities in the Laboratory. Contr. Mar. Sci. 18:19-33.

Hansen, D.J., P.R. Parrish and J. Forester. 1974. Aroclor® 1016: Toxicity to and Uptake by Estuarine Animals. Environ. Res. 7:363-373.

Hansen, D.J., L.R. Goodman, and A.J. Wilson, Jr. 1977. Kepone® Chronic Effects on Embryo, Fry, Juvenile, and Adult Sheepshead Minnows (Cyprindon variegatus), Chesapeake Sci. 18:227-232.

References (Cont'd)

Hansen, D.J. 1983. Utility of Toxicity Tests to Measure Effects of Substances on Marine Organisms. This Volume.

Hansen, S.R. and R.R. Garton. 1982. Ability of Standard Toxicity Tests to Predict the Effects of the Insecticide Diflubenzuron on Laboratory Stream Communities. Can. J. Fish. Aquat. Sci. 39:1273-1288.

Huggett, R.J. and M.E. Bender. 1980. Kepone® in the James River Environ. Sci. Tech. 14:918-923.

Huggett, R.J., M.M. Nichols, and M. Bender. 1980. Kepone® Contamination of the James River Estuary. In Contaminants and Sediments. Ann Arbor Science Publishers, Inc. Ann Arbor, Michigan. 33-52.

Kimerle, R.A., W.E. Gledhill, G.J. Levinskas. 1978. Environmental Saftey Assessment of New Materials in Estimating the Hazard of Chemical Substances to Aquatic Life-Cairns/Dickson/Mahi Editors. ASTM-STP-657.

Lowe, J.I., P.R. Parrish, J.M. Patrick, Jr., and J. Forester. 1972. Effects of the Polychlorinated Biphenyl Aroclor® 1254 on the American Oyster Crassostrea virginica. Mar. Biol. 17:209-214.

Natural Resources Defense Council, et al., v. Train, 8E.R.C. 2120. 1976.

Nichols, M. and R. Trotman. 1979. The Role of Sediments in the Storage, Movement and Biological Uptake of Kepone® in Estuarine Environment: Bed Sediments and Suspended Sediments. 1979. U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida Report. Grant #R804993010.

Nimmo, D.R., R.R. Blackman, A.J. Wilson, Jr., and J. Forester. 1971. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp Peraeus duorarum. Mar. Biol. 3:191-197.

References (Cont'd)

Nimmo, D.R. and L.J. Bahner. 1976. Metals, Pesticides and PCB: Toxicities to Shrimp Singly and in Combination. In Estuarine Processes: I Uses, Stresses, and Adaptation to the Estuary. Academic Press, Doc. New York. 523-531.

Nimmo, D.R., L.H. Bahner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson, Jr. 1977. Mysidopsis bahia: An Estuarine Species Suitable for Life-Cycle Toxicology and Hazard Evaluation. ASTM STP 364, F.L. Mayer and J.L. Hamelink, Eds. American Society for Testing and Materials, 109-116.

Onishi, Y. and R.M. Ecker. 1978. Chapter VII - The Movement of Kepone® in the James River. in The Feasibility of Mitigating Kepone Contamination in the James River Basin. U.S. Dept. Energy Contract EY-76-C-06-1830.

Parrish, P.R. 1974. Aroclor® 1254, DDT and DDD, and Dieldrin: Accumulation and Loss by American Oysters (Crassostrea virginica). Proc. Nat. Shellfish Assoc. 64:7.

Roberts, M.H. and R. Bendl. 1982. Acute Toxicity of Kepone® to Selected Freshwater Fishes. Estuaries, In press.

Schimmel, S.C., D.J. Hansen, and J. Forester. 1974. Effects of Aroclor® 1254 on Laboratory - Reared Embryos and Fry of Sheepshead Minnows (Cyprindon variegatus). Trans. Amer. Fish. Soc. 103:582-586.

Schimmel, S.C. and A.J. Wilson, Jr. 1977. Acute Toxicity of Kepone® to Four Estuarine Animals. Chesapeake Sci. 18:224-227.

Schimmel, S.C., J.M. Patrick, Jr., L.F. Faas, J.L. Oglesby, and A.J. Wilson, Jr. 1979. Kepone®: Toxicity and Bioaccumulation on Blue Crab. Estuaries. 2:9-15.

References (Cont'd)

Stephan, C.E. 1982. Statistical Methods for Calculating Final Acute Toxicity Values. Personal Communication.

U.S. Department of the Interior. Federal Water Pollution Control Administration. 1968. Water Quality Criteria: Report of the National Technical Advisory Committee to the Secretary of the Interior.

U.S. Environmental Protection Agency. 1972. Water Quality Criteria 1972. A Report of the Committee on Water Quality, Environmental Studies Board, National Academy of Sciences, National Academy of Engineering.

U.S. Environmental Protection Agency. 1976. Water Quality Criteria for Aquatic Life.

U.S. Environmental Protection Agency. 1980a. Water Quality Criteria Documents: Availability Federal Register, 45:231. Friday, November 28, 1980.

U.S. Environmental Protection Agency. 1980b. Polychlorinated Biphenyl Criteria Document. EPA-440/5-80-068.

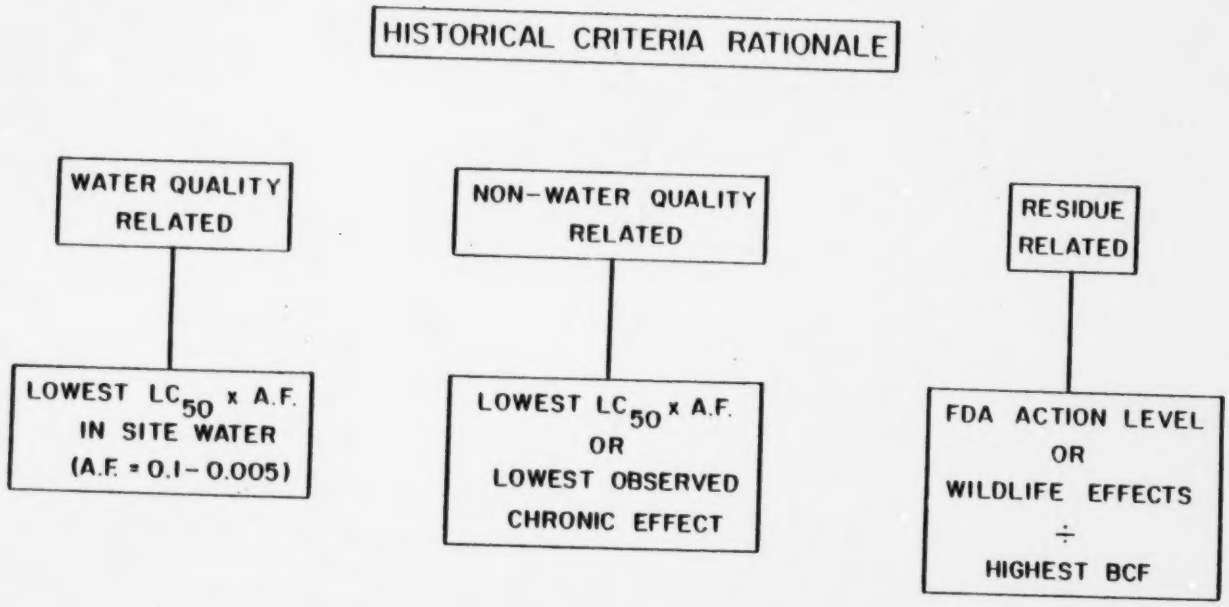
HISTORICAL CRITERIA RATIONALE
WATER QUALITY RELATED
NON-WATER QUALITY RELATED
RESIDUE RELATED
LOWEST LC_{50} x A.F. IN SITE WATER (A.F. = 0.1 - 0.005)
LOWEST LC_{50} x A.F. OR LOWEST OBSERVED CHRONIC EFFECT
FDA ACTION LEVEL OR WILDLIFE EFFECTS ÷ HIGHEST BCF

Figure 1. Three approaches formerly used in the development of Water Quality Criteria from 1968 thru 1976.

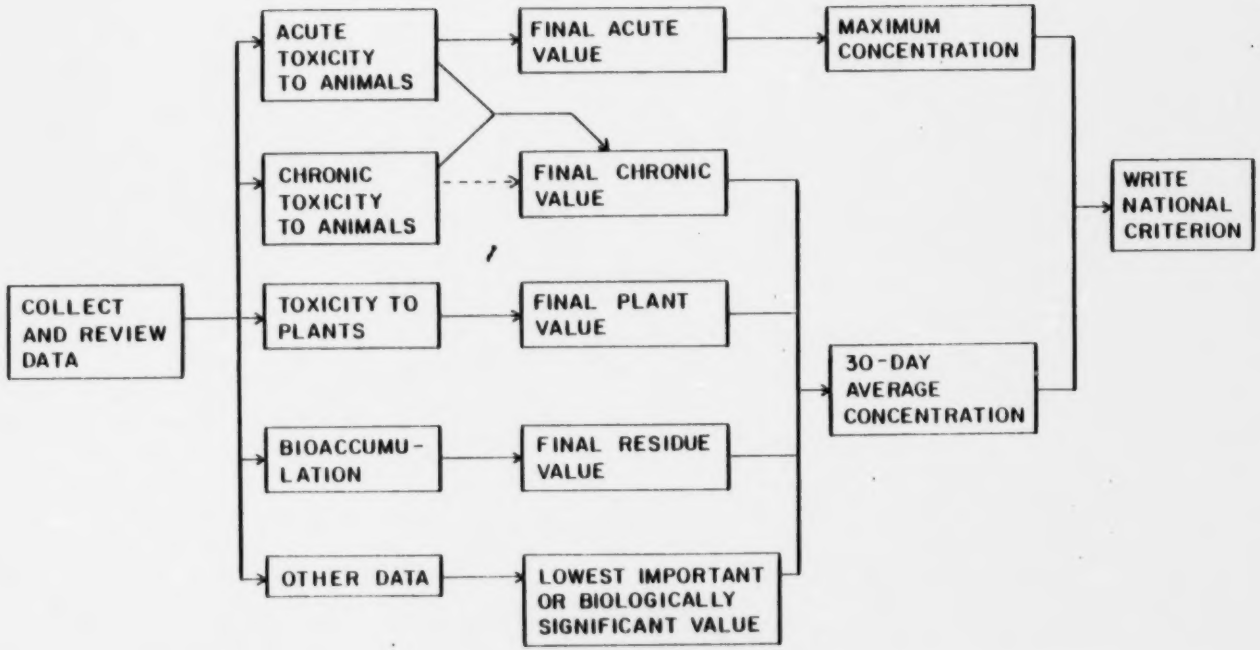

Figure 2. Schematic of the rationale used currently for the development of Water Quality Criteria for the protection of aquatic life and wildlife.

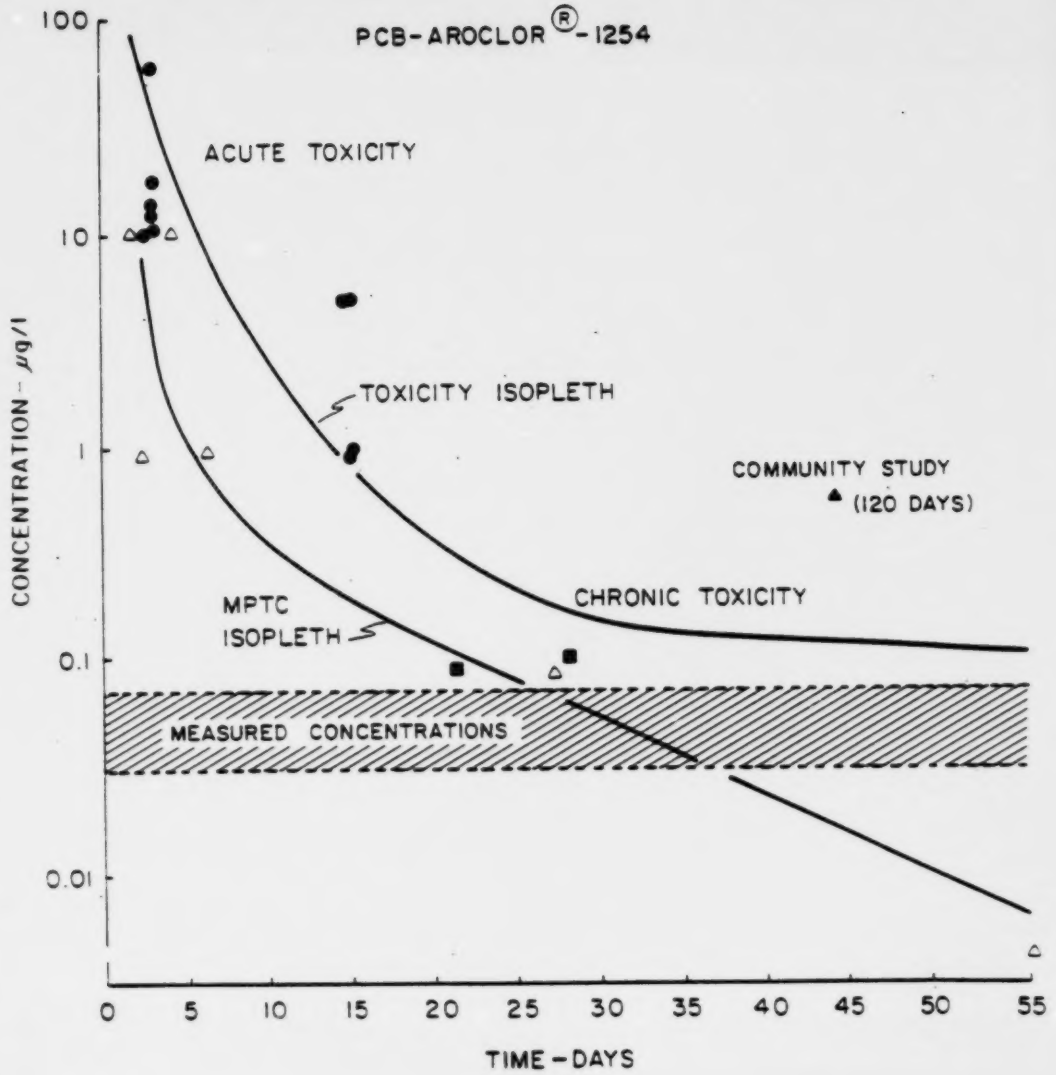

Figure 3. Laboratory-derived toxicity (closed data points) and bio-concentration (open triangles) of the polychlorinated biphenyl, Aroclor® 1254 compared with measured water concentrations of the chemical in escambia Bay, Florida. MPTC is the maximum permissable tissue concentration (5 mg/kg) allowed in fish and shellfish.

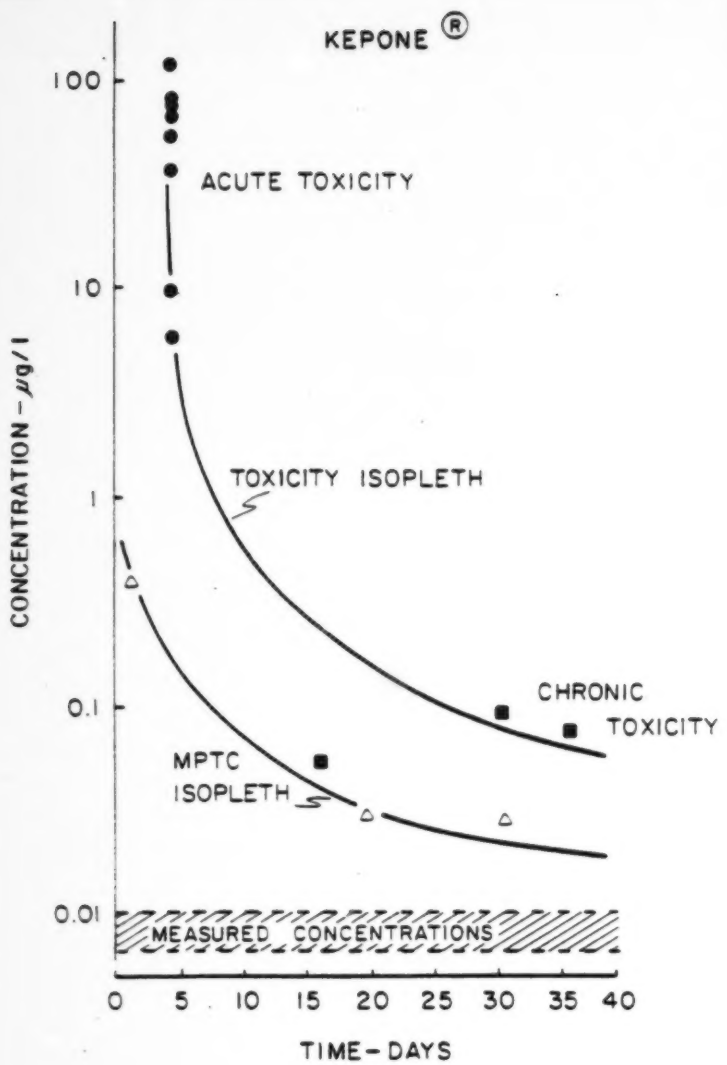

Figure 4. Laboratory-derived toxicity (closed data points) and bio-concentration (open triangles) of the insecticide, Kepone® compared with measured water concentrations of the chemical in the James River Estuary, Virginia, MPTC is the maximum permissable tissue concentration (0.3 mg/kg) allowed in fish and shellfish.